ISSN (print) 0093-4666 © 2011. Mycotaxon, Ltd. ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/118.213

Volume 118, pp. 213–218 *October–December 2011*

Exserohilum neoregeliae sp. nov., a new pathogen of *Neoregelia carolinae*

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Abstract —*Exserohilum neoregeliae* is described from *Neoregelia carolinae* leaves imported from the Netherlands at plant quarantine inspection in Japan. The new fungal species is characterized by long rostrate conidia with an obconic basal end cell with a distinct hilum. ITS + 5.8SrDNA sequence analyses revealed that *E. neoregeliae* forms a monophyletic group with *E. minor*.

Key words — hyphomycetes, *Bromeliaceae,* leaf blight, phylogeny

Introduction

Neoregelia carolinae (Beer) L.B. Sm. (*Bromeliaceae*) is an ornamental plant native to South America (Takabayashi 1994). In May 2006, brown spindle-shaped lesions were found on *N. carolinae* leaves imported from the Netherlands during plant quarantine inspection at Narita International Airport in Japan. A new species of *Exserohilum* isolated from the lesions is described based on its distinctive conidial morphology. In addition, molecular phylogenetic analyses were carried out on the internal transcribed spacer of ribosomal DNA (rDNA-ITS), including 5.8S rDNA.

Materials & methods

Isolates (IM201-c, -d, -e, -f) were obtained from four lesions on the leaves of *N. carolinae* and used for morphological observation, pathogenicity tests, and molecular phylogenetic analyses. For morphological observation, the isolates were incubated on V8 juice agar under alternating 12 h black light blue (BLB)/12 h darkness at 25°C for about 20 days, and the resulting anamorph was observed under light microscopy. Pathogenicity tests were conducted by placing V8 juice agar pieces, including hyphae, on needle-wounded leaves of the original host that was grown in a greenhouse for four

Table 1. Conidial morphology of *Exserohilum neoregeliae* and similar species.

Species	*neoregeliae* [a]	*longisporum* [b]	*rostratum* [c]	*longirostratum* [c]	*minor* [c]
Shape	Narrowly obclavate or rostrate	Fusiform, narrowly obclavate or rostrate	Ellipsoid, narrowly obclavate, or rostrate	Narrowing gradually to long apical beak	Fusiform
Color	Pale brown to brown	Yellowish brown	Brown or olivaceous	Olivaceous brown	Olivaceous brown
No. of distosepta	6–20 (–26)	(6–)8–13 (–18)	≤ 18	—	5–11
Length	80.5–285	54–240	15–200	60–475	55–135
Width	12.5–27	11.5–21.5	7–29	12–26	12.5–20
Septa	Concolorous	Concolorous	Darker & thicker at basal septum	End cells often cut off by dark, thick septum	Concolorous
Base	Basal cell obconic; occ. constricted at/ below septum	Basal cell often sl. bulging	Subhyaline	Broadest at base	Base truncate obconic; lower half occ. constricted

[a] = Isolate No. IM201-D; [b] = Sun et al. (1996); [c] = Sivanesan(1986)

months. The aseptic V8 juice agar pieces were placed in the same way as a control. The inoculated plants were kept in a plastic case with a cover to maintain high humidity for 2 days. Then they were kept at room temperature under natural light after uncovering. The ITS+5.8S rDNA were amplified with ITS1/4 primers (White et al. 1990), sequenced directly, and analyzed to reveal the phylogenetic relationship of the new species with other *Exserohilum* species registered in the DNA Data Bank of Japan (DDBJ; see also Berbee et al. 1999, Chung et al. 2005). The DNA sequences were aligned using ClustalX v2.0.7 (Larkin et al. 2007). Phylogenies were generated using distance methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter method (Kimura 1980) and was analyzed with the neighbor-joining method (Saitou & Nei 1987) using ClustalX v2.0.7 (Larkin et al. 2007). The sequences of isolates IM201-D and IM201-E were registered in DDBJ as AB607956 and AB607957, respectively.

Symptoms

The brown spindle-shaped lesions were usually observed in the center of the leaves (Fig. 1A, B) but occasionally near the leaf margin (Fig. 1C). Some lesions elongated along the veins or turned grayish white with brown margins, producing leaf blight. No sporulation was detected from any lesions under natural conditions.

Taxonomy

Exserohilum neoregeliae Sakoda & Tsukib., **sp. nov.** Fig. 1E–G

MycoBank MB 561917

Coloniae in agaro 'V8 succus' cinereae vel brunneae, floccosae. Hyphae pallide brunneae, ramosae, septatae, laevis. Stromata absentia. Conidiophora singularia, erecta, vel flexuosa,

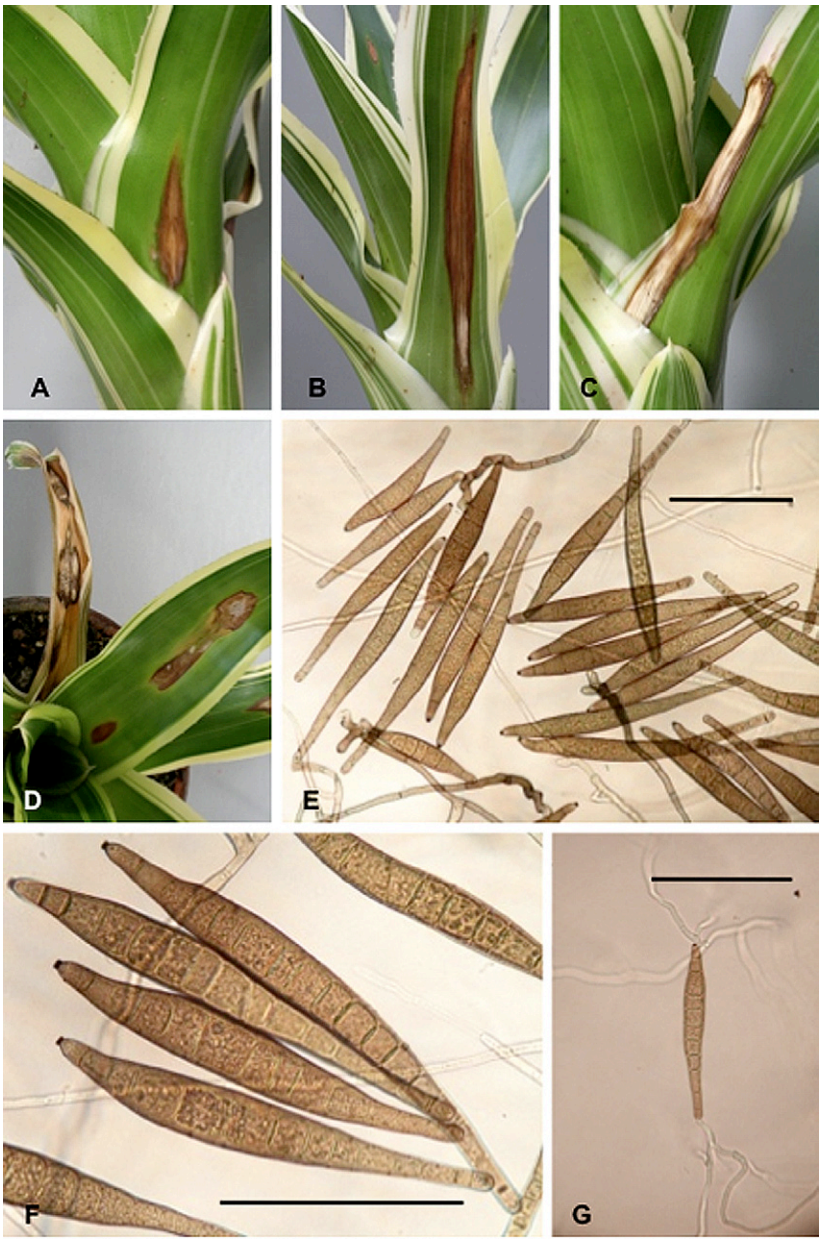

Fig. 1. *Exserohilum neoregeliae*. A–C. Natural symptoms on *Neoregelia carolinae*; D. Symptoms on *N. carolinae* inoculated with IM201-E; E–F. Conidia of IM201-D on V8 juice agar; G. Germinating conidium. Scale bars: E–G, 100 μm.

supra geniculata, laevia vel verruculosa, pallide brunnea, ad 340 µm longa, 5–10 µm lata. Conidia pallide brunnea vel brunnea, concoloria, lepto-obclavata vel rostrata, recta vel leviter curvata, laevia, ad vel infra septum basale constricta, apex obtusus, basis obconica, hilo protrudenti, 6–20(–26)-distoseptata, 80.5–285 × 12.5–27 µm. In foliis Neoregeliae carolinae.

Type: NIAESH 20605 (**Holotype** IM201-D), dry cultura isolated from affected dead leaves, Narita, Chiba, Japan, 24 May 2006, T. Sakoda, in Herbarium of the Institute of National Agro-environmental Science, Tsukuba, Japan. **Isotype:** NIAESH 20606 (IM201-e).

Colonies on V8 juice agar gray to brown, floccose. Hyphae pale brown, branched, septate, and smooth. Stromata not formed. Conidiophore solitary, straight, or flexuous, sometimes geniculate above, smooth to verruculose, pale brown, up to 340 µm long, 5–10 µm wide. Conidia pale brown to brown, concolorous, narrowly obclavate or rostrate, straight or slightly curved, smooth, sometimes constricted at basal septum or below it, apex obtuse, basal cell obconic, with a distinctly protruding hilum, 6–20(–26)-distoseptate, 80.5–285 × 12.5–27 µm (av. 187.2 × 20.6).

Key characters — The conidial morphology of *E. neoregeliae* is similar to that of *E. longisporum* G.Y. Sun (Sun et al. 1997), *E. rostratum* (Drechsler) K.J. Leonard & Suggs, *E. longirostratum* (Subram.) Sivan., and *E. minor* Alcorn (Sivanesan 1987) but does show differences (Table 1). Although concolorous and narrowly obclavate or rostrate conidia are common to both, the conidial basal end cell often bulges slightly in *E. longisporum* but is obconic in *E. neoregeliae*. While the conidial basal end cells are often cut off by thick dark septa in both *E. rostratum* and *E. longirostratum,* in *E. neoregeliae* all septa are concolorous. The conidia of *E. neoregeliae* are broader than those of *E. longisporum* and shorter than those of *E. longirostratum*. The conidia of *E. minor* are not rostrate but fusiform, although the obconic basal end cell is especially similar to that of *E. neoregeliae*.

Pathogenicity

Approximately twenty days after inoculation, symptoms similar to the natural ones were reproduced, and inoculated fungi were readily reisolated from the lesions (Fig. 1D), whereas controls remained symptom-free.

Phylogenetic analysis & discussion

According to the neighbor-joining tree derived from ITS+5.8S rDNA sequences made in this study, the two *E. neoregeliae* isolates (AB607956 and AB607957) form a monophyletic group (100% bootstrap value) close to *E. minor* (AF071341) (Fig. 2). However, the 91.6% (489/534 bp) sequence similarity between *E. neoregeliae* and *E. minor* indicates that those two species clearly differ. Moreover, despite similar conidial morphologies, *E. neoregeliae* is

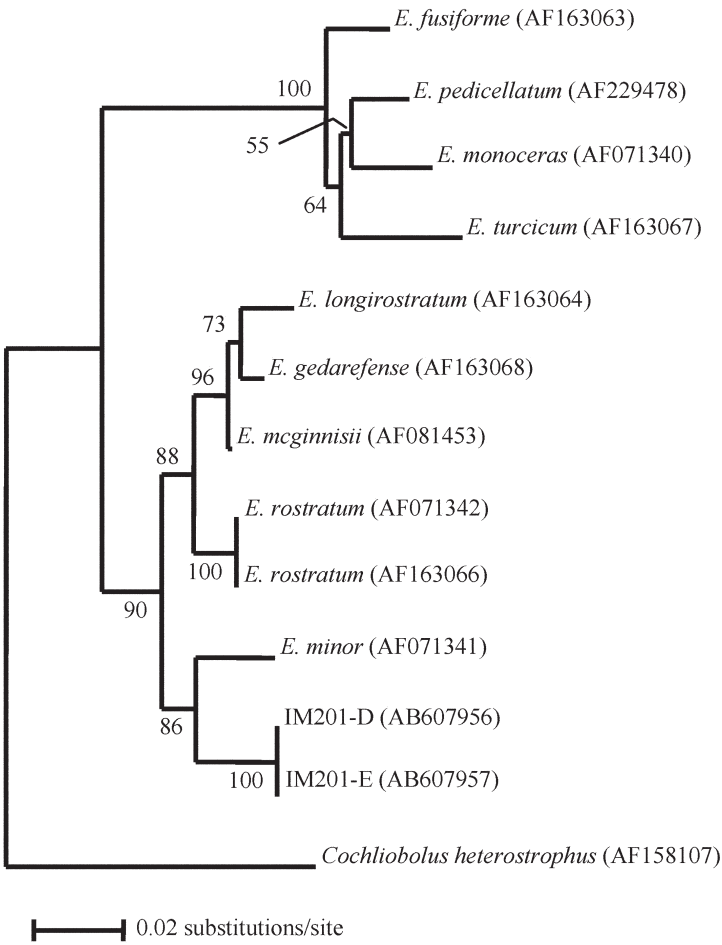

Fig. 2. A neighbor-joining tree inferred from the ITS+5.8S rDNA sequences from 10 *Exserohilum* taxa. The number in front of represented isolates shows the bootstrap values in 1000 bootstrap replicates. The rDNA-ITS accession numbers in DDBJ are shown in parentheses. The length of branches is proportional to the number of base changes, indicated by the scale bar. *Cochliobolus heterostrophus* (AF158107) is outgroup.

in a different clade from *E. rostratum* and *E. longirostratum*. Other *Exserohilum* species with relatively short or non-rostrate conidia, including *E. turcicum* (Pass.) K.J. Leonard & Suggs and *E. pedicellatum* (A.W. Henry) K.J. Leonard & Suggs (Sivanesan 1987), were considerably distant from *E. neoregeliae*. Both

morphological and phylogenetic evidence supports *E. neoregeliae* as a new species of *Exserohilum*.

Acknowledgments

The authors are grateful to Dr. Meng Zhang (Henan Agricultural University) and Dr. Wen-Hsin Chung (National Chung Hsing University) for serving as pre-submission reviewers. We also express our deep thanks to Dr. Shaun R. Pennycook (Manaaki Whenua Landcare Research) for nomenclatural review.

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